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BIOLOGICAL STUDIES ON VX DURING FISCAL YEAR 1958 (U)

F. N. Marzulli
C. L. Punte
J. F. O'Leary

October 1959

Toxicology and Physiology Divisions
Directorate of Medical Research
U. S. ARMY CHEMICAL WARFARE LABORATORIES
Army Chemical Center, Maryland

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Chemical Warfare Laboratories Special Publication 2-18

BIOLOGICAL STUDIES ON VX DURING FISCAL YEAR 1958 (U)

Project No.: 4-08-02-023

APPROVED:

Date Submitted for Publication: 30 September 1958

DOUGLAS LINDSEY
Lt. Col., MC
Director of Medical Research

FOREWORD

The information contained herein was assembled at the suggestion of Dr. D. B. Dill in conjunction with a Research Staff Conference held at the Medical Research Directorate of these Laboratories on 12 March 1958.

The authors are responsible for compilation and organization of the material. The experimental data were obtained by the investigators listed in each section. In time, participant investigators will report their findings separately and in greater detail.

(C)

CONTENTS

[redacted]

(C) INTRAVENOUS TOXICITY OF VX (U)

Investigators: J. F. Callahan, P. Cresthull, M. K. Christensen, J. W. Crook, J. S. Wiles, E. Owens, J. Hart, and F. X. Worden

(C) The intravenous toxicity of VX has been studied in seven species of animals. Results, given in table 1, show that the monkey and pig are the most resistant to the toxic effects of this agent. The cat, goat, and dog are the most sensitive species and the guinea pig and rabbit are intermediate. The LD50 for the monkey, 19.7 μg/kg, is five times that for the cat, 3.8 μg/kg.

(C) Table 1

Lethality of VX by Intravenous Injection in Seven Species of Animals (U)

Injection site	Species	LD50* (19/20 limits)	Chemical purity of agent
		μg/kg	%
Saphenous	Monkey	19.7 (15.4-25.2)	94
Ear	Pig	13.2 (10.1-17.3)	94
Marginal ear	Rabbit	9.4 (8.1-10.9)	94
Saphenous	Guinea pig	7.0 (5.5-8.6)	94
Saphenous	Dog	4.7 (3.8-5.8)	94
Jugular	Goat	3.9 (2.8-5.5)	ca. 90
Femoral	Cat	3.8 (3.2-4.5)	94

* Litchfield-Wilcoxon method of statistical analysis.

[redacted]

[redacted]

(C) Inhibition of RBC-ChE has been studied in seven species of animals following intravenous injection of VX. Results for six species, given in table 2, show that those species most resistant to lethal effects of VX are not necessarily those most resistant to RBC-ChE inhibition. There is a five-fold relation between the doses of VX inhibiting 50% of the RBC-ChE in the most resistant and in the most sensitive species. The value of the ratio LD50/ChE50 for VX is not as consistent as that for GB, ranging between 4 for the dog and rabbit to 15 for the monkey. The RBC-ChE activity in the cat was so low and so variable that a good estimate of the ChE50 was not obtained. A separate study, conducted on cats, showed no relation between the amount of RBC-ChE activity and survival following an LD50 of VX.

(C) Table 2

Anticholinesterase Effects of VX Following Intravenous Injection (C)

Species	ChE50	LD50/ChE50
	µg/kg	
Monkey	1.3	15
Pig	1.2	11
Rabbit	2.1	4
Guinea pig	0.9	8
Dog	1.3	4
Goat	0.4	10

(U) Signs of poisoning displayed by various animals are reported in table 3 as a function of the dose of VX injected intravenously. Signs are quite similar in nature from one species to another, although the order of appearance is not necessarily the same. Cessation of respiration generally occurs in 1 to 1-1/2 hours at the LD50, with the exception of the monkey, which stops breathing in approximately 1/2 hour at this dose. Convulsions and prostration are signs which, when they occur in man, are considered incapacitating. At the LD50, these signs are seen generally within 8 to 18 minutes (table 3).

[redacted]

Table 3

Signs of Poisoning in Various Animal Species Following Intravenous Injection of VX (U)

Species	Dose	Calculated effect for 24-hour observation period	Approximate time for 50% response				
			Tremors	Drooling	Convulsions	Prostration	Cessation of respiration
	µg/kg				min		
Rabbit	6.3	LD34	35	-	-	-	-
	7.9	LD42	23	-	-	-	-
	10.0	1.1 x LD50	6	33	43	18	74
Goat	4.0	LD50	10	10	12	17	60
	6.3	1.6 x LD50	9	9	15	-	50
	10.0	2.5 x LD50	4	6	6	6	38
Dog	5.0	1.1 x LD50	11	16	15	16	65
	6.3	1.3 x LD50	8	12	9	13	42
	7.9	1.7 x LD50	5	7	8	8	24
Cat	5.0	1.4 x LD50	-	25	-	21	26
	6.3	1.7 x LD50	14	-	-	17	21
Monkey	12.6	LD32	4	12	8	16	-
	15.8	LD40	3	10	6	14	-
	20.0	LD50	2	10	4	8	30
	25.0	1.3 x LD50	1	3	1.5	-	29
Guinea pig	6.3	LD46	9	-	-	-	-
	7.9	1.1 x LD50	5	-	15	-	90
	10.0	1.5 x LD50	2	-	4	9	38
	12.6	1.8 x LD50	2	-	4	8	30
Pig	7.9	LD30	6.8	10.2	ca. 15	-	-
	10.0	LD38	3.0	14.0	17.0	17.0	-
	12.6	LD48	3.4	-	11.5	15.5	-

(C) INHALATION STUDIES (U)

Investigators: R. A. Pierce and J. Weimer

I. (C) Aerosol.

A. (C) Stress.

The effect of stress just before the inhalation of VX is being studied in rats. Stress is produced by electrically shocking (100 to 150 volts) the animals for 1 second every 2 minutes over a period of 20 to 24 hours. Within 15 minutes after this period, rats subjected to this stress, along with control rats, were exposed to VX in a Ct range of 20 to 40 mg min/cu m. The Lt50 (lethal time for 50% of the animals responding) for both stressed and control groups was 13 minutes. This indicates that this type of stress has no effect on the speed of action of VX following inhalation. However, the 24-hour mortality fractions for the stressed and control groups were 57/66 and 43/67, respectively. These values are significantly different.

B. (C) Chronic Exposures.

(C) Chronic inhalation studies are being conducted on dogs. Four dogs which were exposed to a Ct of 1 mg min/cu m (1/15 LCt50) for 20 sec/day, 5 times a week, for 12 weeks, showed no significant change in body weight, rectal temperature, BSP retention, prothrombin time, or blood values for lactic acid, pH, CO_2, sugar, potassium, sodium, phosphate, chloride, phospholipids, and NPN. RBC-ChE values on different days fluctuated between 20 and 80% of control values, showing alternate rises and falls. There were no visible toxic signs. Beginning with the thirteenth week, the inhalation exposure was raised to a Ct of 2 to 3 mg min/cu m.

(U) By the end of the thirteenth week, all dogs began to show toxic signs (increased respiratory ventilation, coughing, gagging, apprehension, and some salivation). RBC-ChE values then showed a consistent low level of activity as contrasted with the fluctuating values seen up to the thirteenth week of exposure.

II. (C) Vapor.

(U) That the vapors of VX are toxic has been established previously. However, the conditions under which exposures are made play an important part in the toxicity values obtained. The data summarized in table 4 show that the toxicity of VX vapors to mice in a gassing chamber is greater under static than under dynamic flow conditions.

(C)

Table 4

Effect of Exposure Conditions on Toxicity of VX Vapors to Mice (U)

Exposure time		Ct*	Mortality fraction
Flow	Static		
min		mg min/cu m	
120	-	350-425	0/40
110	10	60-145	1/20
110	10	270	8/13
60	30	150-160	20/20

* As determined by the brom-cresol purple method, J. A. Ph. A. 39, 680 (1950).

[redacted]

(C) PERCUTANEOUS TOXICITY STUDIES (U)

Investigators: A. Van de Wal, D. Payne, J. S. Wiles, C. Myers, J. T. Weimer, and T. A. Ballard

I. (C) Liquid.

A. (C) Bare Skin.

(U) Having obtained more complete information on the inherent (iv) toxicity of VX to various animal species, it has been possible to grade the skin-penetrating potency of VX in different species by means of the ratio pc LD50/iv LD50 (table 5). Results suggest that the skin of the pig may be the most resistant to penetration by VX. The horse, monkey, and rabbit, on the other hand, may be the most sensitive species.

(C) Table 5

Relation Between Intravenous and Percutaneous Toxicities of VX to Several Animal Species (U)

Species	LD50		pc/iv	Hairiness	Dorsal thoracic sweat glands
	iv	pc			
	μg/kg				no./sq cm
Monkey	19.7	63	3	Heavy	40-42
Pig	13.2	320	24	Sparse	10-20
Rabbit	9.4	28	3	Heavy	0
Guinea pig	7.0	ca. 100	14	Heavy	0
Horse	ca. 7	<12.5	1-2	Heavy	>1000
Dog	4.7	50	11	Heavy	45-50
Goat	3.9	20	5	Heavy	50-120
Cat	3.8	<40	<10	Heavy	10-20

[redacted]

[redacted]

(U) The sparseness of hair follicles in the skin of the pig, coupled with the small number of sweat-gland openings, may account for the resistance of this species to percutaneous penetration by VX. The sensitivity of the horse to cutaneously applied VX, on the other hand, may be related to the large number of active sweat glands per unit area in this animal's skin as well as to the numbers and sizes of hair follicles and sebaceous glands therein.

(C) Chronic percutaneous exposures of two dogs were conducted in a manner similar to the chronic inhalation studies already reported. These dogs received 1/10 LD50 of VX or 5 μg/kg, 5 days per week, for 12 weeks. Measurements and results were similar to those reported in the chronic inhalation studies.

B. (C) Clothed Skin.

(C) Quantitative studies have been conducted to find out whether a V agent is capable of traversing the same total distance as efficiently when there is a space to traverse as when layers of clothing are contiguous. Two clothing assemblies were attached to clipped rabbits, each of which provided a 0.1-inch total traversal distance from the outermost to the innermost portion of the clothing. In assembly A, this thickness was provided by three layers of cotton sateen over one layer of cotton underwear. In assembly B, this thickness was provided by one layer of cotton sateen over one layer of cotton underwear, separated by a 0.065-inch spacer consisting of an aluminum ring with 0.62-inch internal diameter. VX was applied to the top layer of each assembly in amounts of 2.0, 0.5, and 0.125 mg/kg and the mortality fractions were observed at 7, 10, 12, and 24 hours in order to calculate Lt50's and approximate LD50's. Data, shown in table 6, show that the LD50's for 24 hours of observation are approximately alike for each condition; however, the Lt50's differ. At the high dose (2.0 mg/kg), the agent is able to traverse the spacer more quickly, the spaced assembly having an Lt50 of 9.3 hours contrasted with one of 17.5 hours for the four-layer assembly. At the 0.5-mg/kg dose, the Lt50 is somewhat lower for the four-layer assembly than for the spaced assembly. One can conclude from this that, practically speaking, there is little difference between the two assemblies, although a space is more readily traversed by VX vapor when the amount of applied agent is relatively high (table 6).

(U) A second experiment was performed to study, in a limited way, the effects of alternate separation and contact, continuous contact, and continuous separation between two layers of cloth. Three clothing assemblies were tested, using clipped rabbits as above. Assembly A

[redacted]

[redacted]

(C) Table 6

Comparison of Toxic Effects of VX to Clipped Rabbits Protected by Four Layers of Clothing or Two Layers of Clothing Separated by a Spacer, Both Clothing Assemblies Having the Same Thickness (U)

Clothing assembly*	Amount of VX applied	Mortality fraction at various observation times				Lt50
		7 hours	10 hours	12 hours	24 hours	
	mg/kg					hr
A	2.0	0/10	1/10	2/10	8/10	17.5
B	2.0	0/10	6/10	8/10	10/10	9.3
A	0.50	0/10	0/10	1/10	6/10	22.0
B	0.50	0/10	1/10	2/10	4/10	28.5
A	0.125	0/10	0/10	0/10	0/10	-
B	0.125	0/10	0/10	0/10	0/10	-

* Assembly A: three layers of cotton sateen over one layer of cotton underwear; total thickness, 0.1 inch; LD50 for 24-hour observation period, ca. 0.5 mg/kg.

Assembly B: one layer of cotton sateen over one layer of cotton underwear, separated by 0.065-inch spacer; total thickness, 0.1 inch; LD50 for 24-hour observation period, ca. 0.6 mg/kg.

consisted of cotton sateen over cotton underwear, separated by an 0.065-inch spacer. Assembly B was the same as A but had no spacer. Assembly C was again the same as A, but alternate contact and separation were provided for 1/2-hour periods during the first 7 hours of exposure.

(C) Results, involving application of 0.5 and 0.25 mg/kg to each clothing assembly, show essentially the same LD50's for a 24-hour observation period. The Lt50's show that, from the viewpoint of penetration,

[redacted]

continuous contact and alternate contact are somewhat superior to continuous spacing. The shorter total traversal distance in assemblies B and C may account for the greater speed of penetration through these assemblies (table 7).

(C) Table 7

Comparison of Toxic Effects of VX to Clipped Rabbits Protected by Three Types of Two-Layer Clothing (U)

Clothing assembly*	Amount of VX applied	Mortality fraction at various observation times				Lt50
		7 hours	10 hours	12 hours	24 hours	
	mg/kg					hr
A	0.5	0/10	1/10	5/10	8/10	14.2
B	0.5	1/10	5/10	8/10	10/10	9.8
C	0.5	1/10	6/10	8/10	10/10	9.6
A	0.25	0/10	0/10	0/10	0/10	-
B	0.25	0/10	0/10	2/10	5/10	24.0
C	0.25	0/10	0/10	1/10	5/10	24.0

* Assembly A: cotton sateen over cotton underwear, separated by 0.065-inch spacer; LD50 for 24-hour observation period, ca. 0.4 mg/kg.

Assembly B: cotton sateen over cotton underwear without spacer (continuous contact).

Assembly C: cotton sateen over cotton underwear, contact and separation of layers alternated with 0.065-inch spacer at 30-minute intervals during first 7 hours; LD50 for 24-hour observation period, ca. 0.3 mg/kg.

II. (U) Aerosol.

A study of 5-micron particles (table 8 and figure 1) of radio-labeled (p^{32}) VX indicates the following:

(1) The deposition of VX aerosols on clipped rabbit skin increases as the wind velocity increases.

(2) The rate of disappearance of VX (about 50 μg), applied as aerosol, from clipped skin of anesthetized rabbits (by evaporation plus absorption) is rapid during the first 2 hours. After that, the rate is considerably slower. The disappearance of VX from sateen cloth, which is attributed solely to evaporation, is at a rather slow rate.

(U) Table 8

Deposition of Radio-Labeled VX Aerosol on Clipped Rabbit Skin (U)

(5-micron particles)

Wind velocity	Relative deposition
mph	
6	1.0
18	2.7
30	3.2

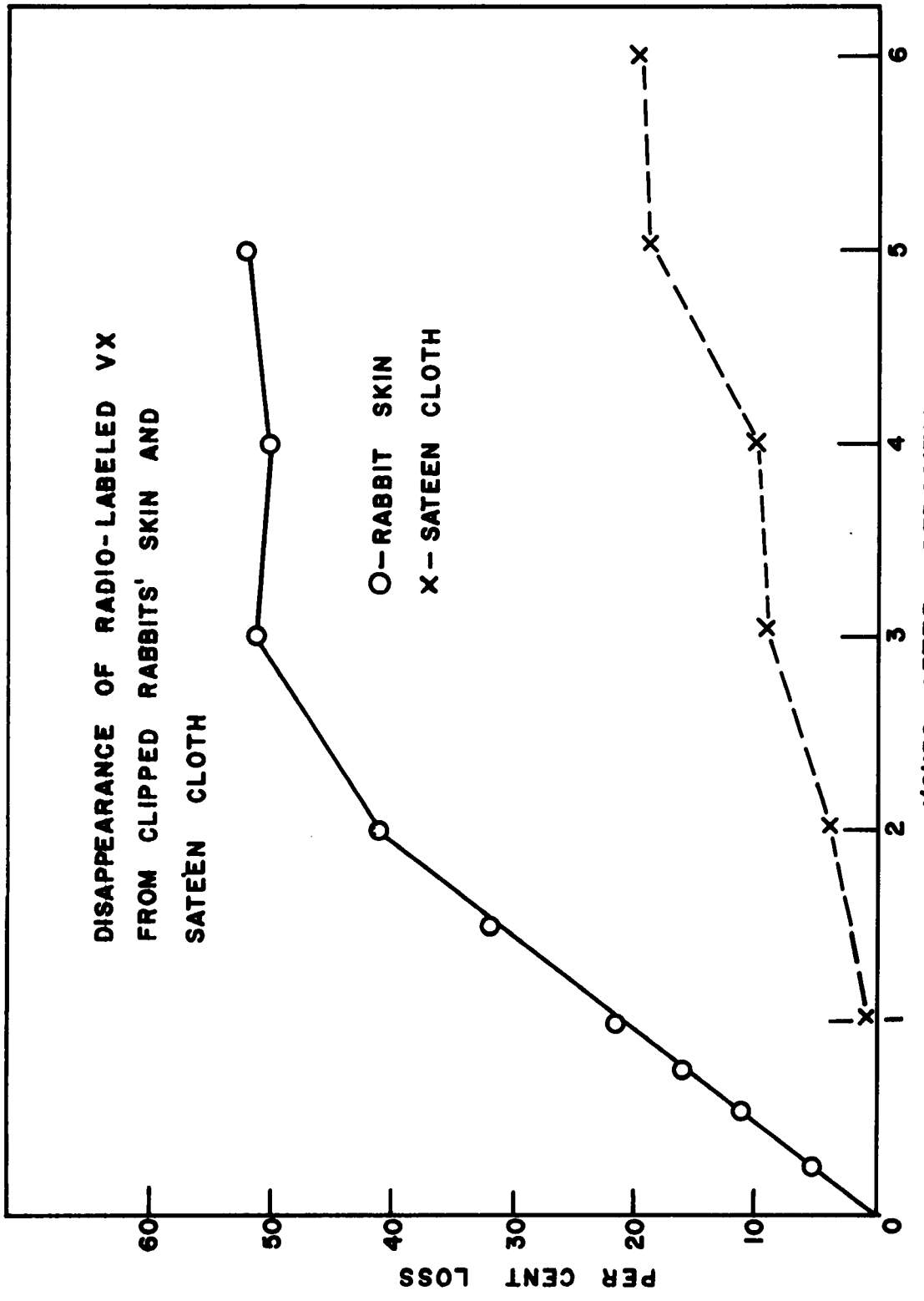

DISAPPEARANCE OF RADIO-LABELED VX
FROM CLIPPED RABBITS' SKIN AND
SATEEN CLOTH
O—RABBIT SKIN
X—SATEEN CLOTH
PER CENT LOSS
HOURS AFTER DEPOSITION
0
10
20
30
40
50
60
1
2
3
4
5
6

FIGURE 1

[redacted]

(C) INACTIVATION AND RECOVERY OF RED BLOOD CELL CHOLINESTERASE (C)

Investigators: R. F. Dunn, J. F. Callahan, and M. Joffe

I. (C) In Vitro Studies.

(C) Effects of various storage conditions on RBC-ChE activity of human blood were studied. A pint of citrated blood which had been stored for 22 days for transfusion purposes was used in this study. The sample was divided, the cholinesterase in each 250 ml being partially inactivated by the addition of 1 μg of VX. Fifty-six aliquots were made, consisting of uninhibited and inhibited samples, refrigerated and unrefrigerated, whole blood and 1:1 cells in saline. The samples were analyzed by the electrometric procedure of Michel over an 8-day period. Results have not been analyzed statistically; nevertheless, variation of values from the initial by more than 0.08 pH units is considered a reasonable index of a significant change. Table 9 shows that, by this criterion, unrefrigerated inhibited samples stored as whole blood or as 1:1 cells in saline appear to show a significant rise in RBC-ChE activity by the fourth day of storage, suggesting that the inhibitory action of VX is being reversed. A significant change appears to take place also in the uninhibited, unrefrigerated samples stored as 1:1 cells in saline. In these samples, a loss in RBC-ChE activity is apparent by the fourth day. Refrigerated, uninhibited whole blood appears also to undergo deterioration of RBC-ChE activity.

(U) Further evaluation of the variables reported in table 9 is shown in table 10. The per cent inactivation of inhibited samples is here reported in terms of the uninhibited values as controls. In this case, using the arbitrary assumption that a 15% change in RBC-ChE activity is significant, one sees an apparent rise in RBC-ChE activity in unrefrigerated samples, by the fourth day. No advantage is seen in storage as whole blood or as a 1:1 saline-cells mixture in unrefrigerated samples.

II. (C) In Vivo Studies.

Recovery of RBC-ChE was studied in six dogs following intravenous administration of VX (0.63, 2.0, and 3.0 μg/kg). Results, given in table 11, show that the recovery rate between the first and eighth day after administration of agent is a straight-line relation when plotted on semilog paper (figure 2, per cent RBC-ChE on arithmetic scale and time in

[redacted]

Table 9

Change in RBC-ChE of Human Blood (ΔpH/hr) Obtained From a Single Source a/ Following Storage of 56 Aliquots as Whole Blood and as a 1:1 Cells-Saline Mixture, Refrigerated and Unrefrigerated and Partially Inhibited by VX and Uninhibited (C)

Pre-storage treatment	Storage conditions	RBC-ChE on various days after storage b/						
		0 day	1 day	4 days	5 days	6 days	7 days	8 days
		ΔpH/hr						
VX-inhibited c/	Refrigerated (5°C)							
	Whole blood	0.455	0.375	(0.335)	0.375	(0.345)	0.430	0.370
	Cells-saline, 1:1	0.455	0.475	0.465	(0.350)	0.525	0.495	0.490
	Unrefrigerated (26-28°C)							
	Whole blood	0.425	0.480	(0.695)	(0.585)	(0.660)	(0.670)	(0.670)
	Cells-saline, 1:1	0.505	0.510	(0.670)	(0.630)	(0.605)	(0.670)	(0.550)
Uninhibited	Refrigerated (5°C)							
	Whole blood	0.985	(0.890)	(0.695)	(0.725)	(0.675)	(0.675)	(0.750)
	Cells-saline, 1:1	1.000	1.010	0.945	(0.905)	0.975	0.950	(0.920)
	Unrefrigerated (26°-28°C)							
	Whole blood	(1.135)	1.020	1.095	(0.895)	0.940	0.920	0.800
	Cells-saline, 1:1	0.965	0.925	(0.765)	(0.635)	(0.730)	(0.655)	(0.740)

a/ Stored as citrated blood to be used in blood transfusions for 22 days prior to use in experiment.

b/ Values within parentheses differ from the original value by more than 0.08 pH unit.

c/ Inhibited by 1 μg of VX per 250 ml of whole blood.

Table 10

The Apparent RBC-ChE* Activity of Blood Samples Reported in Table 9 (U)

Storage conditions	RBC-ChE calculated as per cent of control on various days after storage**						
	0 day	1 day	4 days	5 days	6 days	7 days	8 days
Refrigerated (5°C)							
Whole blood	46.1	42.1	48.2	51.7	51.1	(63.7)	52.2
Cells-saline, 1:1	45.5	47.0	49.2	38.6	53.8	52.1	53.2
Unrefrigerated 26-28°C							
Whole blood	37.4	47.0	(63.4)	(65.3)	(97.7)	(99.2)	(89.3)
Cells-saline, 1:1	52.3	55.1	(87.5)	(99.2)	(82.8)	(102.2)	(74.3)
Average	45.3	-	-	-	-	-	-

* Using uninhibited samples of table 9 as control and inhibited samples as experimental.

** Values within parentheses differ from the original per cent by more than 15 units.

days on logarithmic scale). The rate is rapid at first (4 to 18% per day) but diminishes gradually to 1 to 2% per day as the animal returns to its pre-exposure cholinesterase level. The rate of recovery is also a function of the degree of inhibition, being more rapid following a greater degree of inhibition.

Table 11

Reactivation Rates of RBC-ChE in Dogs Following Intravenous Injection of Various Doses of VX (C)

VX dose	Per cent of control RBC-ChE on various days after injection of VX										Reactivation rates		
	0 day	1 day	2 days	3 days	4 days	5 days	6 days	7 days	8 days	9 days	Day 1	Day 2	Day 8
µg/kg												%/day	
0.63	84.5	86.3	91.7	-	95.8	97.6	97.6	103.6	99.4	-	5	2	1
0.63	89.1	87.0	94.6	-	95.1	97.3	96.7	98.3	98.9	-	4	2	1
2.0	44.2	51.2	67.4	-	74.4	72.7	82.0	84.9	80.8	-	11	5	2
2.0	47.5	56.8	72.8	-	81.4	80.5	86.4	85.6	92.4	-	11	5	2
3.0	21.9	42.1	50.0	62.5	-	-	78.1	79.7	75.0	89.8	18	8	-
3.0	8.9	63.4	81.5	90.4	103.8	-	-	112.7	112.1	113.4	12	5	2
3.0	15.4	35.3	52.5	57.0	67.9	-	-	72.5	83.5	82.5	12	8	3

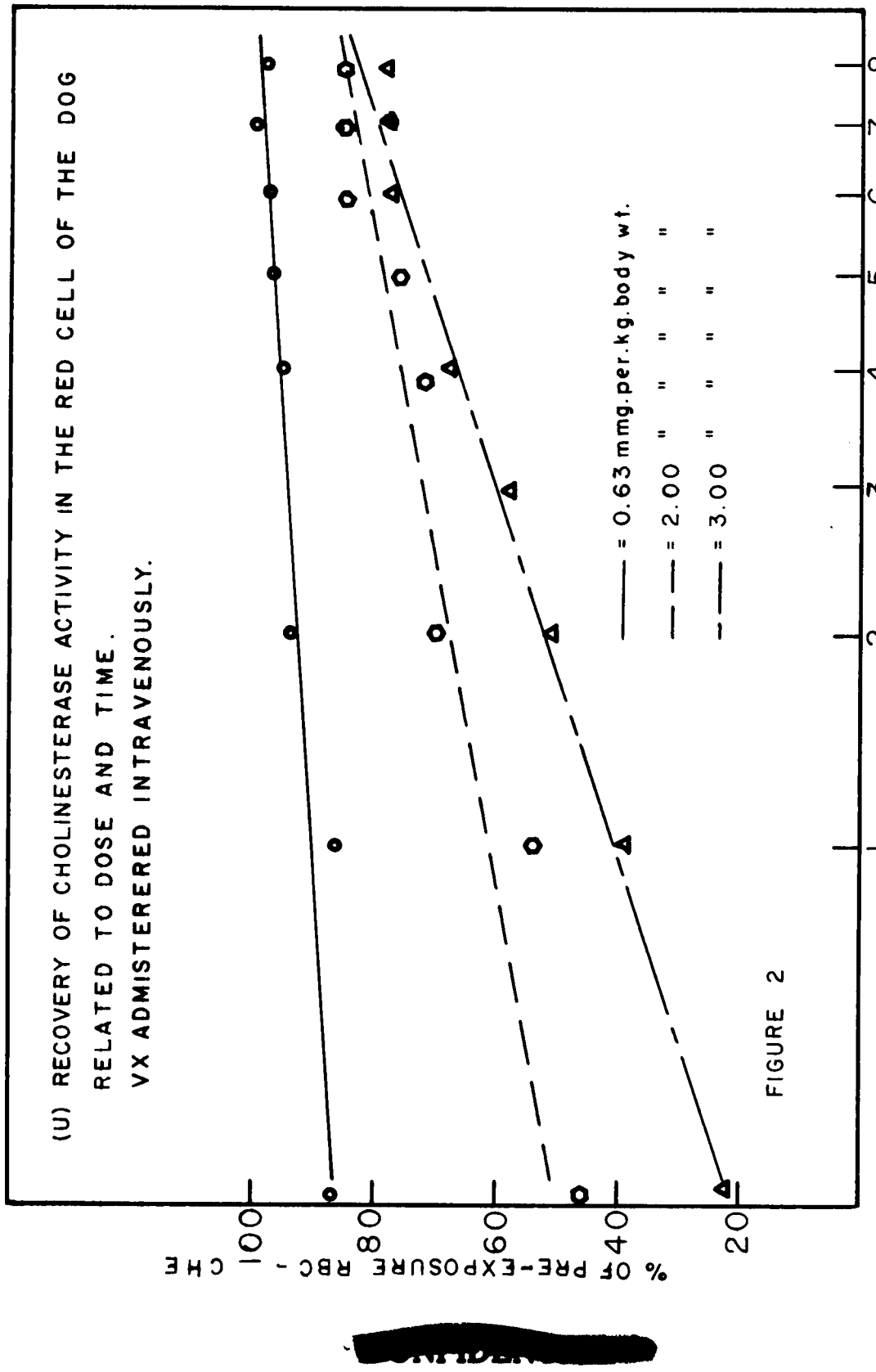

(U) RECOVERY OF CHOLINESTERASE ACTIVITY IN THE RED CELL OF THE DOG
RELATED TO DOSE AND TIME.
VX ADMISTERERED INTRAVENOUSLY.
% OF PRE-EXPOSURE RBC - CHE
100
80
60
40
20
1
2
3
4
5
6
7
8
DAYS AFTER ADMINISTRATION
= 0.63 mmg.per.kg.body wt.
= 2.00 " " " "
= 3.00 " " " "
FIGURE 2

FIGURE 2

[redacted]

(C) DETOXICATION OF VX (U)

Investigators: J. S. Wiles, A. Van de Wal, D. Payne, C. Myers, J. Hart, P. Belluscio, and P. Nowakowski

(U) Detoxication of VX is here defined as the ability to recover from toxic effects. These studies on detoxication involve several methods of evaluation as well as different species and routes of administrations.

I. (C) Acute Studies.

A. (C) Intravenous Route.

(C) In order to measure the amount of agent detoxified over a period of x hours, a sublethal dose of agent is administered followed in x hours by an amount of agent which is known to produce a partial mortality in stock animals. Summation of the two doses and the mortality response enables a calculation of the LD50. Comparison of the LD50 so obtained with that for a single dose of agent gives an estimate of the amount of agent detoxified during the injection interval. By this technique, the amount of intravenously injected VX which was detoxified by rabbits during the first hour, first 4 hours, and first 24 hours was estimated. The rate of detoxication was found to vary as a function of time, being approximately ten times as rapid during the first 4 hours as during the next 20 (table 12).

(U) In order to study the detoxication rate as a function of the dose of agent administered, various sublethal doses of agent were administered intravenously (including one dose which was an LD10). Results, shown in table 13, indicate that detoxication may be a function of the amount of agent injected. Within the range studied, 59 to 80% of the dose administered was detoxified in 24 hours.

B. (C) Subcutaneous Route.

In another detoxication study, mice surviving a subcutaneous LD50 of VX (22 μg/kg) were reinjected with an LD50 on subsequent days. The data, summarized in figure 3, show that mortality, on reinjection after 1 and 2 days recovery, was 100%. Mortality after 8 days, due to reinjection, was similar to that in control animals. This indicates that it takes mice at least 8 days to recover from a subcutaneous LD50.

[redacted]

Table 12

Detoxification Rates of Intravenously Injected VX in Rabbits as a Function of Time (U)

Animal group	Amount administered		Time interval between doses	Mortality fraction	Calculated LD50	Amount detoxified (from change in LD50)		
	Initial dose	Second dose				Total	Average rate per hr for interval	Cumulative
	μg/kg		hr		μg/kg	μg/kg		
1	0.0	9.0	-	18/30	8.7	Ref. pt.	Ref.	Ref.
2	4.0	5.2	1	18/30	8.9	0.2	0.20	0.20
3	4.0	5.4	4	10/30	10.0	1.3	0.37*	0.32
4	4.0	7.0	24	18/30	10.7	2.0	0.035**	0.08

* Time interval of 3 hours (4 minus 1).

** Time interval of 20 hours (24 minus 4).

[redacted]

(C)

Table 13

Detoxification Rates of Intravenously Injected VX in Rabbits as a Function of the Amount Introduced (U)

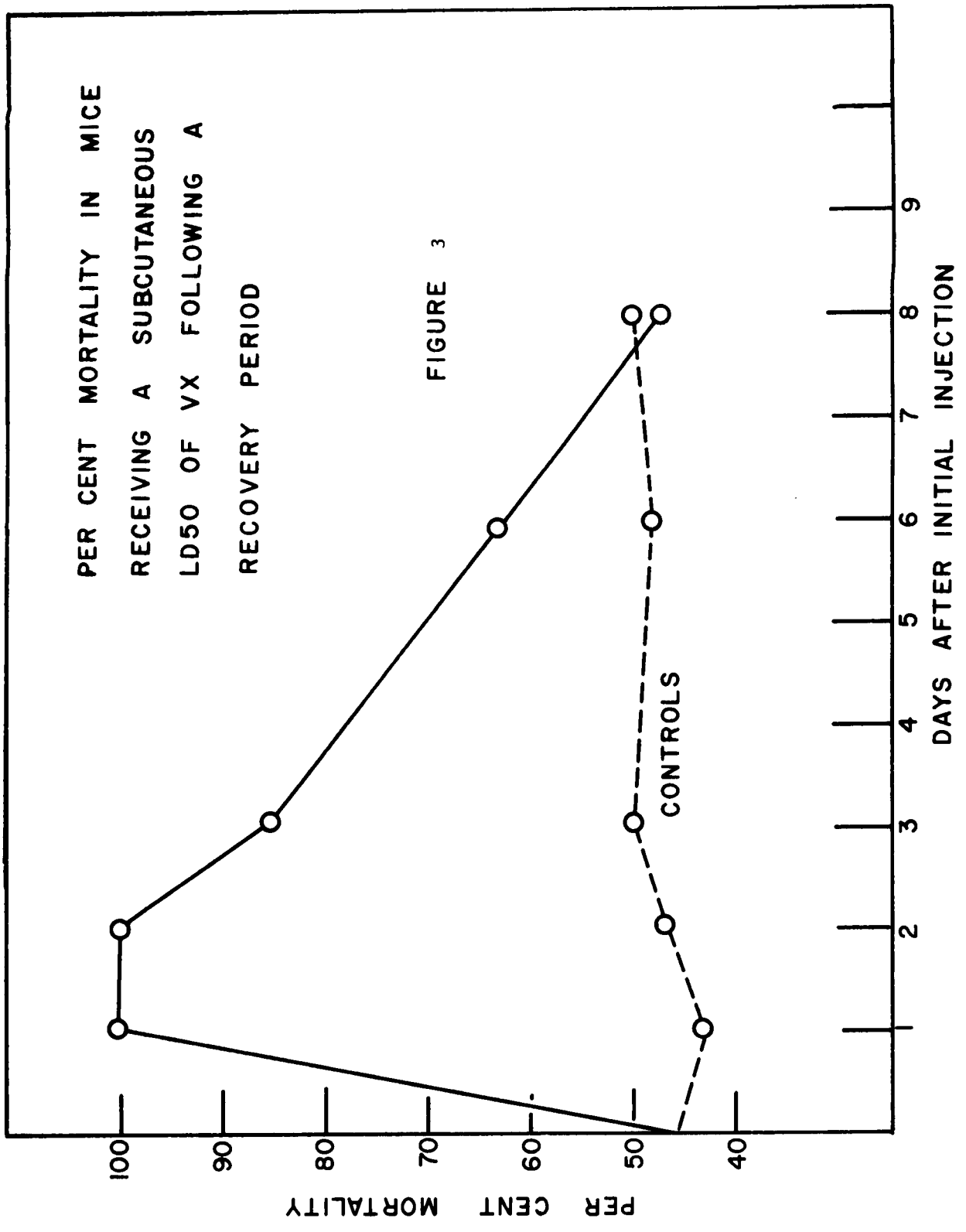
Initial dose	Second dose	Mortality		LD50	Amount detoxified (from change in LD50)		
					Per 24 hr	Per hr	Per cent of initial dose
μg/kg		fraction	%	μg/kg	μg/kg		
0.0	9.0	22/30	73	8.4	-	-	-
2.5	8.4	24/30	80	10.1	1.7	0.07	68
4.0	6.9	10/30	33	11.6	3.2	0.13	80
5.5	5.4	6/30	20	12.1	3.7	0.15	67
6.9	4.0	4/30*	13	12.5	4.1	0.17	59
6.9	4.0	1/30**	3	13.7	5.3	0.22	76

* Includes those killed by initial dose.

** Excludes those killed by initial dose.

[redacted]

[redacted]



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II. (C) Chronic Studies.

A. Intravenous Route.

The effect of daily intravenous administration of VX in rabbits has been studied. The data, summarized in table 14, indicate that daily doses of up to 3 μg/kg produce no toxic signs in rabbits over a period of 23 days. However, doses of 4.5 μg/kg, or higher, produce toxic signs and death within 16 days. These data indicate that the average detoxication rate for VX injected rapidly in rabbits is about 0.12 μg/kg per hour.

(C) Table 14

Effect of Daily Intravenous Administration of VX in Rabbits (U)

Dose	Days	Fraction showing toxic signs	Mortality fraction
μg/kg			
0.6	7	0/4	0/4
2	13	0/4	0/4
3	23	0/4	0/4
4.5	16	4/4	4/4
6.5	3	4/4	4/4
7.5	2	4/4	4/4

In another study, two dogs received rapid intravenous injections of VX on 5 days per week, for 5 weeks. A dose of 0.5 μg/kg in one dog and 1 μg/kg in other dog produced no toxic signs. Both dogs were then given 2 μg/kg daily for 3 additional weeks, without toxic signs. This indicates that the average detoxication rate for rapidly injected VX in dogs is at least 0.08 μg/kg per hour.

[redacted]

[redacted]

B. Percutaneous Route.

Two dogs exposed percutaneously to 5 μg/kg of VX for 5 days per week, for 12 weeks, showed no toxic signs. When the dose was raised to 10 μg/kg, toxic signs (increased respiratory ventilation, coughing, gagging, apprehension, and some salivation) developed within a week. At the end of the seventeenth week, RBC-ChE values for one dog had fallen to 5% of normal. This suggests that the percutaneous detoxication rate is greater than 0.4 μg/kg per hour.

C. Slow Infusion Study.

When 10 μg/kg of VX is administered intravenously to rabbits at different rates, from very rapid to 0.08 μg/kg per minute, mortality fractions are different. The data, summarized in table 15 suggest that when VX is administered at a slow rate, about 1 to μg/kg per hour, are detoxified. To verify this detoxication rate, studies are now underway in which rabbits receive a dose of 1.5 μg/kg per hour for a 6-hour period. After this 6-hour period, an LD50 is injected rapidly into the animals and mortality observed at 24 hours. On the basis of results with 6 experimental and 12 control animals, there appears to be little difference in mortality between the two groups.

(C) Table 15

Effect of Rate of Intravenous Administration of VX on Toxicity in Rabbits

Dose and rate of administration	Mortality fraction
10 μg/kg, rapid	7/9
10 μg/kg/hr	4/19
10 μg/kg/2 hr	0/10

[redacted]

[redacted]

Table 16 is a summary of the detoxication rates for VX obtained in these studies. In six of the seven studies, the average rate of detoxication is about 0.1 μg/kg per hour. In one, involving slow infusion of agent, the rate appears to be significantly higher, amounting to as much as 1 to 2 μg/kg per hour. When administered at this slow rate, the animal may be capable of detoxifying almost all of the agent injected.

(C)

Table 16

Summary of Detoxification Rates of VX Obtained by Different Techniques

Species	Route	Method	Detoxification rate
			μg/kg/hr
Rabbits	iv	Acute, rapid injection	0.07-0.32
		Chronic, rapid injection	0.12
		Slow infusion	1-2
Mice	sc	Recovery test	0.12
Dogs	iv	Chronic, rapid injection, 5 days/wk	0.04-0.08
	pc	Chronic, 5 days/wk	>0.4

[redacted]

(C) EFFECTS OF ACCLIMATIZATION (U)

Investigators: J. A. LeBlanc, L. E. Gongwer, and C. Punte

I. (C) Inhalation Studies.

(U) Exploratory studies of the effect of acclimatization to low temperature on toxicity of VX to rats have been initiated. Results, summarized in table 17, suggest that acclimatization at 40°F for 1 or for 28 days does not appreciably change the toxicity of VX by inhalation as determined in control animals at 73°F.

(C) Table 17

Effect of Acclimatization at 40°F on the Inhalation Toxicity of VX in Rats

Ct	Mortality fraction of rats acclimatized for various periods before exposure		
	0 day	1 day	28 days
mg min/ cu m			
27	8/9	15/17	8/17
34	7/9	17/17	11/17
43	-	8/8	7/8

II. (C) Percutaneous Studies.

The Lt50 for rats exposed on the paws to [illegible] mg/kg of VX at 73°F is about 1 hour; all animals are dead within 2 hours. By contrast, rats acclimatized for 28 days at 40°F exhibited no toxic signs during a 4-hour observation period after application of VX (1 mg/kg). At 18 hours, only 25% of the acclimatized rats were dead.

[redacted]

(C) EFFECTIVENESS OF OXIMES AND OTHER AGENTS AGAINST VX POISONING (U)

Investigators: J. F. O'Leary, R. V. Brown, A. M. Kunkel, A. Oikemus, S. Okerman, L. Somers, D. Sullivan, and F. X. Worden

A systematic consideration of agents which are effective against VX would involve, first of all, atropine. It is apparent that atropine acts, in general, against VX, as against other anticholinesterases, by counteraction of muscarinic effects and by its favorable effect on the central respiratory mechanism. This report will deal mainly with protective effects which can be achieved with other compounds, particularly the oximes 2-PAM and TMB-4 (EA 1814), acting in combination with atropine.

Table 18 is presented to emphasize the main actions of the oximes in supplementing the action of atropine. Anesthetized cats received 0.5 mg/kg of atropine sulfate intravenously, followed in a few minutes by 0.2 mg/kg of VX intravenously. The data show that if the animals receive no further treatment, skeletal muscle response to exitation by its nerve remains at least 50% below normal for the duration of the 3-hour experiment, and, what is more important, the animal cannot breathe unassisted for a period of 1-1/2 hours. If, however, 2-PAM (5 mg/kg) or TMB-4 (10 mg/kg) is given about 5 minutes after the VX, the muscle twitch response returns promptly and reinstitution of breathing follows soon after. But the test muscle is still unable to maintain a half-normal tension, even though the respiratory muscles are functioning adequately; TMB-4, in contrast to 2-PAM, will quickly restore the ability to maintain tension, but this ability quickly fails again and can be restored only by repeating the TMB-4 dose. Thus, it is evident that the return of spontaneous respiration is to some extent associated with return of the twitch response after oxime treatment, but not at all with the tension response, even though the muscular activity of respiration is usually thought of as a tension type of response. To further confuse the picture, return of respiration is dissociated from even the twitch response when atropine alone is the treatment. This situation has led to the suggestion that the oximes may have effects on central respiratory processes, as well as on peripheral neuromuscular transmission. The presence in these drugs of the quaternary nitrogen atom militates against the acceptance of this hypothesis. At any rate, the central fact is that the oximes are able to restore vital respiratory function and herein, so far as we now know, lies their value in counteracting the effects of VX.

[redacted]

(C)

Table 18

The Effect of Oximes on the Reinstitution of Spontaneous Respiration in Cats After VX, as Compared to Recovery of Neuromuscular Response

(Anesthetized cats received atropine sulfate, 0.5 mg/kg, iv, followed in a few minutes by VX, 0.2 mg/kg, iv)

Oxime and dose (iv)	Recovery time		
	Twitch height 50%	Tension 50%	Spontaneous respiration
None	>180	>180	89
2-PAM, 5 mg/kg	6.5	>180	18
TMB-4, 10 mg/kg	6.2	>180*	9

* TMB-4, in contrast with 2-PAM, will quickly restore the ability to maintain tension, but this ability soon fails and can only be restored by repeating the TMB-4 dose.

[redacted]

To consider further the effect of the oximes on respiration, and hence on survival, table 19 is presented. Dogs received 150 μg/kg of VX. Ventilation failed promptly but was maintained artificially until atropine or TMB-4, or both, were given 20 minutes after the VX. Table 19 shows that either atropine or TMB-4 alone did not effect the reinstitution of spontaneous respiration; all animals so treated died. The mixture of atropine and either oxime was in every case effective in restoring respiration and allowing the animal to survive to the end of the experiment several hours later.

Table 20 shows that the oxime-atropine mixture is effective against subcutaneously-administered VX even at quite high dose levels, whereas atropine alone is not.

[redacted]

(C) Table 19

The Efficacy of Late (20 Minutes) Treatment of 20 LD50's of VX by Atropine and Oximes in Anesthetized Dogs

Treatment and dose (iv)	Resumed respiration		Survivors
	Temporarily	Permanently	
Atropine sulfate, 0.5 mg/kg	3/5	0/5	0/5
TMB-4, 10 mg/kg	2/3	0/3	0/3
Atropine sulfate, 0.5 mg/kg, and TMB-4, 10 mg/kg	-	3/3	3/3
Atropine sulfate, 0.5 mg/kg, and 2-PAM, 5 mg/kg	-	6/6	6/6

Doses: A = 0.5 mg/kg; T = 10 mg/kg; P = 5 mg.kg.

(C) Table 20

The Efficacy of Atropine-Oxime Treatment Against VX Given Subcutaneously in Unanesthetized Dogs

Treatment and dose (iv)	Survival after 5-min therapeusis	
	15 LD50's	30 LD50's
Atropine sulfate, 2 mg/kg	0/3	-
Atropine sulfate, 2 mg/kg and 2-PAM, 5 mg/kg	-	4/6
Atropine sulfate, 0.5 mg/kg, and TMB-4, 5 mg/kg	-	3/6

[redacted]

(C) APPENDIX

SUGGESTIONS AND APPLICATIONS (U)

I. (C) Intravenous Toxicity.

A. (U) The dose-response curve for intravenous toxicity of VX to animals is exceedingly steep. Caution is therefore required in the administration of increasing doses of VX to humans.

B. (U) One one-hundredth of the iv LD50 for the most sensitive animal species is a safe exploratory dose for man.

C. (C) The amount of VX which produces a 15% inhibition in RBC-ChE in man (three consecutive readings over a 1/2 hour period) is expected to be safe for human exposures.

D. (U) Additional studies of poisoning are required on man and animals to find out the effects of small doses of VX.

II. (U) Inhibition of RBC-ChE.

Blood taken for RBC-ChE determinations should be refrigerated promptly after removal from animals and man. Individual determinations are less reliable than two or more which are in agreement.

III. (C) Detoxication.

Animals recover from the toxic effects of VX in about 8 days. RBC-ChE may require as much as 2 weeks or longer for recovery, depending on the degree of inhibition. Experiments on man should take these factors into account.

IV. (C) Therapy.

Animal studies indicate that 2-PAM would be a valuable adjunct to atropine in the treatment of anticholinesterase poisoning in man.

[redacted]

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REPLY TO
ATTENTION OF

JAN 06 2017

RDCB-DSR-S

MEMORANDUM THRU Director, Edgewood Chemical Biological Center, (RDCB-D/Dr. Joseph L. Corriveau), 5183 Blackhawk Road, Aberdeen Proving Ground, Maryland 21010-5424

FOR Defense Technical Information Center (DTIC), 8725 John J. Kingman Road, Ft Belvoir, VA 22060-6218

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3. The point of contact is Adana Eilo, ECBC Security Specialist, (410) 436-2063 or adana.l.eilo.civ@mail.mil.

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ECBC Documents for Downgrading/Change in Distribution

1. Callahan, J.F. *The Relation between Skin Thickness and the Penetration Rate of VX through Skin.* In *Research Program of the Field Toxicology Branch;* CRDL TM 20-27; Callahan, JF, *et al.* Eds.; Directorate of Medical Research, U.S. Army Chemical Research and Development Laboratories, U.S. Army Chemical Center: Edgewood Arsenal, MD, 1962; UNCLASSIFIED Report. **CBRNIAC-CB-118810 Dist. E.**
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3. Frankel, H.M.; Wiles, J.S. *Lethality of VX in Rats at High and Low Temperatures;* CRDLR-3023; U.S. Army Chemical Research, and Development Laboratories: Edgewood Arsenal, MD, 1960; UNCLASSIFIED Report **AD0243462 Dist C.**
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8. Sim, V.M; Stubbs, J.E. *VX Percutaneous Studies in Man*; CRDLR-3015; Chemical Research and Development Laboratories: Aberdeen Proving Ground, MD, 1960
AD0318533 Dist. F. Recommended for public release.